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RECENT STUDIES OF THE OLIVE-TUBERCLE ORGANISM.

BY

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INTRODUCTION.

In recent years the olive orchards of California have suffered considerably from swellings on the branches, known as olive tubercles and attributed to bacteria. This disease was first described in Italy, at least in modern times, albeit imperfectly as regards its cause. It has been known since Roman days.

DEPARTMENT EXPERIMENTS.

A desire to learn the natural methods of infection and the exact biology of the organism in the hope that this might bring to light some method of coping with the disease led the writer, in 1903, to begin a systematic study of the olive tubercle, which has continued to date, material being obtained from California and subsequently from Italy. Mr. James Birch Rorer was associated with the writer in the earlier work.

The right organism was plated out of the tumors without difficulty and determined to be a schizomycete. Numerous inoculations by needle puncture were made on sound olive trees in one of the hothouses of the Department of Agriculture with very satisfactory results. During this study it was learned that a yellow schizomycete, perhaps more than one, quite frequently accompanies or follows the parasite, and that occasionally nonpathogenic white bacteria also occur in the tumors. These yellow and white organisms were determined to be nonpathogenic by needle-puncture inoculations. All of the inoculations were made on young growing shoots of the olive. No fungi were found in the tubercles used for the poured-plate isolations, but subsequently fungi were found in some of the old tumors.

During this year (1903) tumors were produced on about 50 olive plants by pure-culture inoculations (on every one inoculated), and with pure cultures plated therefrom a second crop of knots was produced, thus complying with Koch's rules of proof. Many control

punctures on the same plants healed promptly. During the entire seven months only one accidental infection occurred, and this one could be accounted for satisfactorily, i. e., it appeared lower down on a branch already inoculated and forming a tumor. Such being the results, no reasonable doubt remained as to the possibility of producing the tumor with pure cultures of bacteria, nor from our studies was there any doubt as to the particular organism involved. Our experiments fully confirmed Savastano's statements respecting the bacterial origin of the tumor, but the parasite was white, not yellow as stated in literature. These facts were set forth in December, 1903, in an address before the Society for Plant Morphology and Physiology, illustrated by stereopticon and by inoculated plants, an abstract of the same being published in Science.

During the four years which have elapsed since this preliminary note, many additional successful inoculations have been made (the total number of inoculations now exceeding 500), and occasional accidental infections have also appeared on the experimental olives, all of the plants being in one place from the start. These latter, however, have been comparatively few and in no way confusing. About 150 olive plants of several varieties were used for these experiments. They were rooted cuttings from sound plants which had been grown in one of the hothouses of the Department of Agriculture for many years.

OTHER RESEARCHES.

About the same time as the writer, Ruggero Schiff, subsequently known as Schiff-Giorgini, began experimenting on the olive tubercle in Italy. His first paper was published in German in 1904. This was followed in 1905 by a second paper in German and by a longer paper in Italian, wherein he furnished additional evidence of the same character as that given in his first paper. Doctor Schiff claimed to have obtained successful infections with a schizomycete totally different from that isolated in our laboratory. He also stated that cultures of the *Bacillus oleae* are flocculated or agglutinated by juice from tubercles, but not by juice from sound parts of the plant. Fortunately, he described his organism so carefully that no doubt remains as to exactly what he meant by it.

In 1905 the writer called attention to the discrepancy between his own and Schiff's studies, stating some of the points of disagreement, and venturing the statement that no olive knots could be produced with pure cultures of Schiff's organism.

About this time Dr. Amedeo Berlese, an Italian, became interested in knots on olive trees; distinguished, incorrectly it would seem, two distinct forms of tubercle; and cultivated from the ordinary form a yellow organism, inclined to orange, supposed by him to be *Bacillus oleae*

(Arc.) Trevisan, and from the other form a white organism said by Petri to be Schiff's bacillus.

Moreover, in 1905, Peglion, in Italy, described a tubercle from the oleander, noted its anatomical resemblance to the olive tubercle, isolated a schizomycete therefrom, with characters like *Bacillus oleae* (Arc.), and made inoculations, but did not wait for results before publishing. This organism, isolated by Peglion, is unlike the olive-tubercle organism, and in Doctor Petri's experiments and our own, a culture of it having been given me by Doctor Petri, also proved non-pathogenic to the oleander.

In 1906 Clayton O. Smith, of California, also described the oleander tubercle, cultivated a schizomycete therefrom, secured inculations on oleander and olive, and definitely identified the organism as *Bacillus oleae* (Arc.) Trevisan, the growth of this organism on potato being described as spreading, raised, and always straw colored. Peglion's work is not mentioned.

One of Doctor Schiff's colleagues, Dr. Lionelle Petri, assistant to Doctor Cuboni in Rome, took up the subject in 1905, by this time a rather confused one. After experimenting for a period of two years he published a paper in German in 1907, in which he states that both Schiff-Giorgini and Berlese are wrong, the only infectious organism of the three studied by him being that isolated and described by Smith in 1905. The *Bacillus oleae* (Arc.) Trevisan is a mixture. On agar both elements of this mixture may remain alive for some time, or one may destroy the other.

INOCULATIONS WITH SCHIFF'S BACILLUS.

My note of 1905 in the Centralblatt did not cover all my knowledge of the olive tubercle. I included in this note only enough to show that Schiff-Giorgini and myself were experimenting with entirely different organisms and that probably both of us could not be right. I had not then experimented with his organism, but I assumed that Schiff was wrong because we had produced fine examples of the olive tumor repeatedly (50 of them in 1903 and a great many since) with my organism, while he passed over the etiology of his organism with a few inconclusive remarks, to wit:

> It is unnecessary to dwell on the pathogenicity of *B. oleae;* it has already been demonstrated by others, as I said above in speaking of the work already extant on this subject. In addition I will say that I have repeatedly caused the tuberculosis in healthy olive plants, using pure cultures of *B. oleae* in glycerinated broth.

This is all Schiff has to say on inoculation experiments in his paper of 28 quarto pages. In the earlier German papers he barely mentions the subject. I did not consider Berlese's note of any importance, because he did not confirm his statements by successful inoculations.

At the time of my note to the Centralblatt in 1905, I had not seen Schiff-Giorgini's organism, but I obtained it toward the end of 1905 from Kràl, who had it from Kornauth, and made inoculations with it on olive shoots in 1905 and 1906.

All of these inoculations failed, although they were several times repeated, for the most part on young, actively growing shoots, using young cultures and inoculating very copiously with needle pricks on varieties of olives very sensitive to the knot. Altogether about 50 inoculations in three series (including 500 or more punctures) were made with this organism at considerable intervals during a period of about two years, using slant-agar for the first lot, potato cultures for the second, and slant-agar for the third. Not a single tumor developed. In one or two cases only there was a slight swelling in the pricked area such as check punctures sometimes give, but none of these developed into knots, and they were only slight cicatrization elevations. Bacteria were present at the end of a year in some of these punctures, as determined by a microscopic examination of sections. Possibly these slight wound reactions are what Schiff-Giorgini identified as tubercles, for he states that he obtained the disease with "pure cultures" of his organism repeatedly (ripetutamente); or it may be that his inoculation experiments were made with mixed cultures (they were fluid cultures at any rate); or, finally, it may be that they were made on limbs of olives already infected with the tubercle organism and ready to develop tumors. Tubercles on olive trees being more easily observable, if they had been present, than mixtures of bacteria in his fluid cultures, the second supposition is probably the correct one, since Schiff says that the olive trees used were sound ones and that the inoculations were from "glycerinated broth." My twelve olive plants were under examination for many months, and in some instances, especially in the final inoculations, a much greater amount of Schiff's organism was inserted than it has ever been my custom to use in the case of the true tumor-producing organism.

I can therefore confirm Doctor Petri's statement respecting this organism, my own inoculation experiments being perfectly in accord with his. The Schiff-Giorgini organism, which has various well-marked characteristics and is not likely to be mistaken for any other organism peculiar to the olive tree, is not the cause of the olive tubercle, and Schiff's conclusions, therefore, as to immunity, resistance, agglutination; etc., can not be accepted.

There can be no doubt, I think, that I had Schiff-Giorgini's organism, since, except in some slight details likely to vary from culture to culture and person to person, it corresponded perfectly with his own very full description, as I took care to find out before making many

inoculations. Kràl wrote to Schiff for it, who said he did not have it any longer, but perhaps Professor Kornauth would still have it, as proved to be the case.

AGGLUTINATION TEST.

The writer repeated the agglutination test described by Schiff, using young bouillon cultures of the right organism and the juice of a fresh large knot mixed with barely sufficient water to enable one to filter it satisfactorily through a Chamberland bougie. The peptone was thrown out of solution by this fluid, but the bacteria continued separate and motile; they were not in any way clumped by one or two hours exposure or even by exposure over night, nor when one part of the bacterial fluid was put into as much as ten parts of the juice and examined in a hanging drop. Moreover, even if Schiff had had the right organism, twenty to forty hours is altogether too long to wait for an agglutination.

SAPROPHYTIC BACTERIA IN TUBERCLES.

I find it difficult to believe that Schiff-Giorgini's organism occurs in all natural olive knots or that there is any symbiotic relationship. As already stated, saprophytic bacteria frequently occur in olive tubercles, and Doctor Petri touches on the possibility of symbiosis, without, however, committing himself to it. My original belief was that Schiff must have obtained his organism from the surface of an imperfectly sterilized potato culture or from the surface of the olive tubercle, but from Doctor Petri's observations we must now conclude that it occurs in the interior of some of the tubercles. The yellow organisms called *Ascobacterium luteum* by Doctor Petri may occur in all old knots, since we may assume them to be common on the surface of olive trees, just as certain nonpathogenic forms are common on the skin of animals, but they are not by any means always in young knots. In certain poured plates where I found yellow bacteria in conjunction with the right organism I had good reason to think the surface of the tubercle had been insufficiently sterilized (thirty seconds). Neither Schiff's organism nor the yellow form isolated by Berlese is necessary for the production of the tubercle, as Doctor Petri has also shown. In Petri-dish poured plates made from olive tubercles collected in autumn and winter, or in early spring before new growth has commenced, probably also in late summer, one might expect to find various intruding bacteria, also fungi. I have found the bacteria very frequently, especially the yellow ones. Schiff's organism I have not, so far, found in the tubercles. In two instances I have obtained only the nonpathogenic yellow organisms from olive tubercles, but these were at least 6 or 8 months old, and perhaps older—too old

anyway. One lot came from Naples and the other from Tunis. Schiff's mistake arose from the belief that anything found in the tubercle must necessarily be the cause of it. That he did not discover and correct his mistake later on is due to the fact that his inoculations were not properly controlled.

NOMENCLATURE OF THE OLIVE-TUBERCLE ORGANISM.

The nomenclatorial vicissitudes in connection with the olive-tubercle organism may be stated as follows:

1886. *Bacterium oleae* Arcangeli: Bacteria seen in cavities in the tubercles and name given without measurements or any description, except the statement that they resembled *Bact. termo.* The cavities also contained mycelium and spores. No bacteriological studies were made. Statement hazarded that the bacteria had nothing to do with the production of the tubercles which arise in wounds. The tubercles were described quite carefully and attributed to physiological disturbances favored by special predisposition.

1889. *Bacillus oleae tuberculosis* Savastano: Studies begun in 1886; tubercles produced in 1887 and again in 1889 with cultures of bacteria obtained from the knots. Organism described as a motile short rod, which is whitish or yellow on culture media and sometimes liquefies gelatin, while at other times it does not.

1889. *Bacillus Prillieuxianus* Trevisan: A name based on Prillieux's account of the disease and given without study or proper description.

1889. *Bacillus oleae* (Arc.) Trevisan: A simple transfer to another genus. The description is borrowed (curtailed) from Savastano. *B. Prillieuxianus* is placed under it as a synonym; also *B. oleae tuberculosis.*

1904–1905. *Bacillus oleae,* the cause of olive tubercle, stated by Schiff-Giorgini to be a white to dirty yellow, polymorphic, peritrichiate bacillus, producing spores very readily, forming long chains and tangled threads suggestive of the anthrax organism, producing a prompt pellicle on bouillon, liquefying gelatin, coagulating milk, growing readily at 37° C., withstanding a temperature of 102° C., etc.

1905. The olive-tubercle organism stated by Erwin F. Smith to be a white, nonsporiferous, nonliquefying organism, consisting of short rods, motile by means of polar flagella, unable to grow at 37° C., killed by a temperature of 50° C., incapable of coagulating milk, etc. Yellow and white nonpathogenic bacteria stated to occur in the tubercles.

1907. *Bacillus oleae* (Arc.) Trevisan, stated by Petri to be a "Sammelname," which splits up on study into *Bacillus oleae α* (Smith), the

true parasite; *Bacillus oleae β* (Schiff-Giorgini), a nonpathogenic organism; and *Bacillus oleae γ*, which in Berlese's cultures is *Ascobacterium luteum*, a nonpathogenic, liquefying peritrichiate form, and in Savastano's cultures is *A. luteum*, mixed with Smith's organism, an assumption believed to be required by the fact that Savastano describes his organism as sometimes liquefying gelatin and as yellow on some media and yet obtained tubercles on making inoculations.

Such being the situation, by what name shall the olive-tubercle organism be known?

The earliest name, Arcangeli's, is practically a nomen nudum, since the description accompanying it does not presuppose pathogenicity nor enable anyone to determine what organism was intended. Arcangeli's entire description is as follows:

> But I constantly found between the irregular cavities placed more or less deeply (whose walls were oftentimes more or less chestnut colored and changed) colonies of a bacterial form like *Bacterium termo*, which I shall call *Bacterium oleae*, together with mycelial filaments, possibly of a Cladosporium, and spores of fungi.

Savastano was the first man to isolate bacteria from the olive tubercle and to secure infections. His name is the next earliest, but it is a trinomial, and a part of his description is drawn from mixed cultures. The organism does not liquefy gelatin and is not yellow.

Trevisan's names are inadmissible because he merely copied earlier vague statements and no one can tell from his descriptions what organism was intended. This is shown admirably by the very diverse interpretations which have followed, e. g., Berlese's and Schiff's. Indeed, the subject is in such confusion that in recent years no less than four distinct organisms have been considered to be *Bacillus oleae* by different writers.

After careful consideration, to avoid further confusion, the writer has decided to give an entirely new name to the organism isolated by himself, and, in order that Savastano may not fail of due honor, he has decided upon the name *Bacterium Savastanoi*, with the following characterization:

DESCRIPTION OF THE OLIVE-TUBERCLE ORGANISM.

Bacterium Savastanoi (nov. nom., nov. descript.)

Synonym (pro parte): *Bacillus oleae tuberculosis* Savastano.

Names of doubtful import, to be rejected: *Bacterium oleae* Arc.; *Bacillus Prillieuxianus* Trev.; *Bacillus oleae* (Arc.) Trev.

Latin diagnosis.—Baculis cylindricis apicibus rotundatis, longitudine variantibus, solitariis vel in filamentis brevibus dispositis; baculis unis saepe 1.2–3×0.4–0.8 μ; se moventibus, aerobiis, asporis.

Hab.: In tumoribus Oleae europeae. Coloniae in gelatina tenues, albae, marginibus inaequales, nonliquefacientes. Coloniae in agar-agar albae, rotundae, nitentes, evolventes lente (in extremo die septimo 2–5 mm. latae). Culturae in tuberibus Solani primo albae, dein pallidae fulvae-albae sunt. Lac sterile alcalinum fit et casein non-segregatur. Baculi methodo Gram noncolorantur. Nitrum non-redigitur. Acidum in mediis cum saccharo uvae celeriter fit. Si culturae novae in infusione carnis ¼ horam in temperatura 50° C. tenentur, moriuntur. Inter temperaturam 35° C. et temperaturam 1° C. crescit. Inoculatum in Oleas sanas, tumores proprios producit.

A white, nonliquefying schizomycete, causing olive tubercle. This organism is found in the olive tubercle and in many culture media as a short rod with rounded ends, either single or in pairs, growing end to end, or in small clumps, more rarely in short chains; the rods taken from the interior of an unruptured tubercle measured 1.5 to 3 × 0.6 to 0.8 μ when stained by carbol-fuchsin and washed in water; the rods from young agar cultures stained with carbol-fuchsin usually measure about 1.2 to 1.5 × 0.5 μ; rods from 3 days' old colonies on agar measured 1.2 to 2 × 0.4 μ when stained by a modified Gram, i. e., washed in amyl alcohol, and cleared in xylol; more rarely they are 10 μ or more in length; in bouillon kept for three days at 30° C. the thinly clouded fluid examined in hanging drops contained numerous actively motile rods five to twenty-five times as long as broad, some of the long rods plainly constricted in the middle, others not, the long ones flexuous;[a] the organism is motile by means of one to several polar flagella, often 2 to 4 (Pitfield's flagella stain), the rods so stained being 2 to 5 × 0.6 to 0.8 μ.

Young motile agar cultures stain readily with carbol-fuchsin, but not by Gram; the organism is aërobic and very sensitive to heat; the growth is white in various culture media (bouillon, peptone water, milk, standard agar, sugar agars, gelatin (especially at bottom of slant), silicate jelly, starch jelly, Cohn's solution, Fermi's solution, Uschinsky's solution); on +15 nutrient agar in poured plates made from bouillon and incubated at 20° C. or 25° C., the surface colonies are small, circular, smooth, glistening (internal structure reticular), rather slow growing, appearing at the end of the second and third days and best observed after three to four days, becoming denser and whiter with age;[b] the intruders usually come up first in plates made

[a] In a repetition made in 1908 the same result was obtained: The longest single rods were 3 μ; the chains varied from 6 to 40 μ; some of the chains were actively motile.

[b] In +15 standard nutrient agar in Petri-dish poured plates made recently and kept at 22° to 23° C. the surface colonies in thin-sown plates were pure white, rather slow growing (1.5 to 3 mm. at the end of the third day), round, flat, surface smooth, glistening, edge entire or nearly so, internal structure under the compound microscope amorphous to finely granular; buried colonies quite small. At the end of seven days

from the tubercles, and often the right colonies are then best observed after four to six days; bouillon is thinly clouded, and there is finally a small amount of white precipitate—in four days at 20° C., no rim, pellicle, or flocculence; on peptone water a white pellicle after some

the surface colonies were 2 to 5 mm. in diameter and more or less viscid, the slime sometimes stringing up 1 cm., the margin was undulate and there was often a ring. In another set of agar plates made some days later and studied with equal care, the surface colonies were 0.7 to 1.3 mm. in diameter after forty-eight hours at 23° C. The margin was entire. The surface was wet-shining and smooth. The internal structure under the hand lens was reticular. Under the compound microscope it was amorphous to finely granular. The buried colonies were small biconvex. At the end of seven days the surface colonies were white, circular, ringed or not. The surface was smooth and wet; the internal reticulations were still visible under the hand lens. Under the 16 mm. and 12 ocular the colonies were finely granular.

In +10 gelatin poured plates made at the same time, the surface colonies were 1.5 to 2.5 mm. in diameter at the end of four days at 21° C. They were white, slow-growing, round, flat, with undulate, erose, paler white margins, and fine internal striæ. There was no liquefaction. After some weeks the margins were decidedly lobed.

On gelatin the marginal growth is quite characteristic and distinct from the body of the growth, both in colonies and in streaks. The edges of the streaks and colonies are often more or less lobate or incised. Savastano observed this and likened the appearance to that of a leaf. Whenever the slime runs down from the streak it is seen to be white, but often the growth on gelatin is so thin that the yellow color of the gelatin shows through and might thus lead to confusion.

Often after some days a brownish stain is produced on potato and in the fluid and this modifies the color of the bacterial growth, but it can scarcely be called yellow; perhaps tawny or tawny-white comes nearest to describing it. A similar color occurred in the older zoogloeæ forming the rim of two flask cultures containing river water, calcium carbonate, Witte's peptone, and grape sugar.

In undisturbed test-tube cultures in water containing 2 per cent Witte's peptone, a thin white surface membrane forms after some days (five or six) and falls readily as a unit, being broken up only by rather vigorous shaking.

Two water solutions of copper sulphate were prepared, i. e., 1:100,000 and 1:500,000. One-half cubic centimeter of a young, thinly clouded bouillon culture was pipetted into 10 c. c. of each one of these two coppered waters. Checks were held by pipetting one-half cubic centimeter of the same bouillon culture into each of two tubes containing 10 c. c. of pure water. Petri-dish poured plates were made with carefully measured quantities of the fluid at the end of one-half hour, one hour, two hours, and twenty-four hours. Check plates from the pure water dilutions were also made at the end of two hours and twenty-four hours. In all 36 plates were poured (three of each coppered water at each date and 12 check plates). Result: No distinct reduction in colonies by one-half hour, one hour, or two hours exposure; marked reduction after exposure for twenty-four hours. In the 1:100,000 solution *four-fifths* of the bacteria were destroyed. There was also a similar marked reduction in the weaker solution of 1:500,000. The colonies on the plates made from the coppered waters came up a day sooner than those on the check plates, the growth of those bacteria that survived being stimulated. By using a young bouillon culture rather than washings from an agar streak it was believed that the advantage would be in favor of the bacteria, since we may suppose some part of the copper would be rendered inert by the organic matter of the bouillon.

days; it blues litmus milk, the fluid, which is lavender or lilac on the start, becoming gradually a deep blue; it does not form acids in milk and does not coagulate milk by a lab ferment; it grows readily in Cohn's solution and for a long time, producing an abundance of crystals of ammonium magnesium phosphate, no difficulty being experienced in obtaining enough for a chemical analysis from small flask cultures; in old gelatin streaks and in old gelatin stab cultures there was a thin white surface layer, but no trace of liquefaction, growth along the line of the stabs was not well developed; in gelatin streaks and gelatin colonies there is an irregular undulatory lobed or incised margin; organism nonsporiferous, nonliquefying, nongas forming; growth on cooked potato at the end of two days was smooth, white, wet-shining, distinct, but not very copious, cylinder slightly grayed and fluid at the bottom moderately cloudy, the growth at first on potato being not unlike the pure white growth of *Bacillus trachiphilus*, even in old cultures the growth is never a decided yellow (see footnote); potato cultures eighteen days old gave a deep purplish blue reaction when mashed in iodin water, the checks giving a bright blue color, i. e., the organism acts but slightly on potato starch; action on olive starch unknown; it does not reduce nitrates in peptonized beef bouillon; it will not grow in beef bouillon at 38.5° C., and is killed in +15 standard beef bouillon by exposure in the water bath, in test tubes, for ten minutes at 50° C.; streak cultures on litmus agars containing filtered river water and Witte's peptone, but free from beef juice, behave as follows:

Plain litmus agar.—This becomes blue and remains so (forty days), growth moderate.

Litmus dextrose agar.—This reddens promptly (twenty-four hours, at 27° C.) and decidedly, and remains acid indefinitely (color bright red—not purple), growth slight.

Litmus galactose agar.—This reddens promptly and decidedly and remains acid (forty days); same reaction as with litmus dextrose agar, growth moderate.

Litmus saccharose agar.—This remains neutral or nearly so for some days, finally, however, becoming purplish and then purple-red; growth stimulated, i. e., a much greater volume of bacterial slime than on the other agars and the litmus reduced in the bottom of the tube after some days.

Litmus lactose agar.—This blues; growth not more than on the plain litmus agar; no acid was formed (thirty days).

Litmus maltose agar.—This blues, growth moderate; no acid was formed (thirty days), and there was no reduction.

Litmus mannit agar.—This blues, growth moderate, after ten days litmus purplish, never red.

Litmus glycerin agar.—This remains neutral or nearly so, i. e., in nine days only a trace of purpling and no marked increase of red in next twenty days, good growth.

The most striking reaction is the prompt complete reddening of the litmus in the presence of dextrose or galactose and its persistency.

A slight indol reaction (pink color) was obtained with sodium nitrite and sulphuric acid both in peptone water and in Uschinsky's solution with peptone, but it was less than half as deep as that obtained with *Bacillus coli* and it did not form as promptly;[a] the organism has lived in agar in the ice box upward of four months; it grows in +15 bouillon at 1° C. and would probably cloud the fluid at 0° C.; 60 to 90 per cent of the rods were killed by freezing (liquid air); it clouds bouillon over chloroform.

In flask cultures in river water containing 2 per cent Witte's peptone and 2 per cent dextrose, there was very copious growth, but most of the bacteria were dead at the end of six weeks, probably because there was a decided acid reaction, in spite of the presence of a small amount of calcium carbonate. The fluid bore floating islands, but no continuous pellicle. Free oxygen is necessary for the production of this acid, i. e., in fermentation tubes containing filtered river water, Witte's peptone, and dextrose or galactose, the clouding, which was prompt and copious, was confined to the bulb and the outer three-fourths of the U, with a sharp line of demarcation where the bacterial growth ceased. Six tubes were inoculated, three of each sugar, and all behaved alike. There was not a trace of clouding in the closed end up to the end of the sixth day, when the experiment was broken off. The organisms from California and Italy behaved alike. The mixed fluid from the open end of the three bulbs containing grape sugar was acid to litmus paper and titrated +41 on Fuller's scale, using phenolphthalein and sodium hydrate: that from the closed end was neutral to litmus and titrated +20 with phenolphthalein and sodium hydrate. The mixed fluid from the three bulbs containing galactose titrated +30; that from the closed end +20. There was no loss of acidity on boiling thirty minutes.

The organism is sensitive to sunlight. In thin sowings in six Petri-dish poured plates in +15 standard agar, exposed on ice, bottom up, to bright sunlight for thirty minutes in April, the covered one-half of each plate promptly developed from 100 to 150 colonies; the exposed one-half of each plate, on the contrary, remained entirely free from colonies (eight days). The temperature did not exceed 18° C.

It appears to be sensitive to acids. The organism is sensitive to copper sulphate (see footnote).

[a] This was repeated in 1908 in peptone water with what appeared to be a negative result, but on standing some days the fluid in the culture tubes became pink, while that in the check tubes did not. These cultures were ten days old.

When inserted by needle punctures into young growing leaves or shoots of the olive, it causes the characteristic galls known in Italy as *rogna* or *tuberculosis* of the olive, and in California as *olive-knot*, *tumor*, or *tubercle*. In my inoculation experiments it has required three to four months for the tubercle to become full grown, and nine to fifteen days for the appearance of elevations on the stem of unmistakable tuberculous character. Earlier than this it has not been possible macroscopically to detect the incipient tubercles with any certainty. Inoculations are most successful when made on the tips of undeveloped shoots in active growth. It is impossible or difficult to induce the formation of tumors on slow-growing or dormant tissues. The organism is most easily recovered in pure culture by making poured plates from the interior of young knots which have not developed fissures, the surface being first sterilized for five minutes in 1:1,000 mercuric chlorid water. The organism does not lose its virulence readily by continued culture in the laboratory, but I have seen some indications of such loss, i. e., slow development of tumors and a larger proportion of failures.

The disease occurs in Italy, France, Spain, Algeria, Tunis, California, etc., injuring the olive and sometimes destroying it. The organism enters the plant, so far as yet known, exclusively through wounds, and sets up an extensive hyperplasia involving various tissues. I failed to obtain the disease by spraying a water suspension of a young virulent culture upon an actively growing olive shoot, kept in moist air under a bell jar. Moisture in tiny drops persisted on the leaves and stem for a good many hours, but no tubercles resulted (only one experiment, however). The bacteria occur at first between the cells and in small irregular closed pockets, but as the tubercle grows it splits open, foreign organisms appear, and decay sets in. Metastasis occurs, i. e., there are two distinct types of tubercles, primary and secondary, the former due to external infection and beginning in the cortex, the latter due to internal infection and beginning deep in the tissues at the junction of wood and pith.

METASTATIC TUBERCLES.

Metastasis was discovered by Schiff-Giorgini. This was observed and studied by him on natural infections. Recently the writer has also observed it in 10 secondary tumors on shoots and leaves of the 16 inoculated plants referred to below as checks on the oleander inoculations. On one of the leaves the outbreak of the metastatic tubercle, which was watched from its incipiency and before it ruptured the epidermis, appeared on the midrib 4 cm. above the point of inoculation, which was on the stem. On one of the shoots it appeared at a distance of 7 cm. above the primary tubercle. These

secondary tumors are not due to the migration of host cells, but are the result of migrations of the bacteria, which set up local irritations where the secondary tumor arises. The bacteria make their way from the point of inoculation by way of the vascular system. They are easily observed in some portion of the vascular system, usually a very small portion of it, at points anywhere between the primary and the secondary tubercle. In the cases I have studied they were confined to small canals in the inner wood next the pith, these canals being due to the disorganization of a group of vessels. The bacteria were abundant and the walls of the canal were stained yellow and brown. The giving way of the woody structure and the flooding out of the bacteria into softer tissues is apparently what determines the appearance of a secondary tubercle at any particular spot. By splitting the stem lengthwise in the proper place one can trace the canal of infection leading from the primary tumor to the secondary one as a small stained line at the inner border of the wood, easily visible to the naked eye. Sometimes the bacteria are numerous enough in the canal to form a slight ooze on cross section.

THE INOCULATION OF OTHER PLANTS.

This organism apparently is not infectious to *Nerium oleander*. Only six oleander plants were tried, but these very thoroughly, i. e., 18 sets of punctures on as many young actively growing shoots, using agar streaks forty-eight hours old and inoculating very thoroughly, a total of about 150 needle punctures being made. These plants were under observation for five months. No tumors developed. Sixteen olive shoots were held as checks on these oleanders and developed 16 groups of tubercles corresponding to the points of inoculation, and also subsequently metastatic tubercles, as above mentioned. At present, therefore, I am unable to explain the counterstatements of Clayton O. Smith. The oleander tubercle seems to me to be due to *Bacterium tumefaciens* Smith and Townsend.

Apparently the organism is not infectious to *Chrysanthemum frutescens*, i. e., at the same time that the oleanders and olives above referred to were inoculated, actively growing shoots of 12 white daisy plants were inoculated by needle puncture from the same set of cultures. About 120 needle pricks were made and the inoculation was done with great thoroughness, but no tumors developed (five months). *Bacterium tumefaciens* would have produced visible results on the same plants in five to seven days and large tumors in two months.

The above-mentioned sets of inoculations were made in 1907. Earlier in the course of experimentation numerous attempts were made to inoculate the olive disease into ash trees of several species,

privet, and other plants more or less closely related to the olive, but none of the experiments were successful. I still believe it might perhaps be inoculated into *Fraxinus ornus*, my experiments with this species being limited in number and cut short by an accident. Certain slight swellings were visible at the time the plants were destroyed, but the results were not assured. Schiff-Giorgini states that the disease does not occur on the wild olive.

SCHIFF'S ORGANISM A POTATO BACILLUS.

Schiff's organism is *Bacillus vulgatus* (Flügge) Migula or some closely related form, possibly a form near *Bacillus pseudanthracis* Kruse. This will become evident from the following considerations.

SCHIFF'S OWN STATEMENTS.

The principal statements of Schiff respecting the morphology and cultural characters of his organism are condensed as follows from his Italian paper, the starred sentences being observations which the writer has confirmed, using the cultures received from Kornauth:

The organism occurs in the form of long chains or tangled filaments which are usually nonsporiferous, nonmotile, and on staining are seen to be composed of short rods with square ends.* There are also numerous short motile rods with rounded ends, and these in great numbers change quickly into endospore-bearing bodies when grown in culture media.* Transitions occur, the chains becoming shorter and disappearing in old cultures.* Short rods sometimes contain minute irregular granules.* These rods are greenish transparent.* In young cultures on agar, forms occur with and without polar staining. The motile short rods with rounded ends when taken from agar measure 1.6 to 2.2 × 0.6 to 0.8 μ, or from young cultures 2.5 × 0.9 μ. On sugar agar they measure 2 to 3 × 0.8 μ. In broth they are 2 to 3 × 0.8 μ, or when in chains the segments are 1.8 to 2 × 0.7 μ. The organism stains by Gram.* We have, therefore, short rods, short chains, long chains, and pseudo filaments, the ends rounded or truncate, and the elements motile or nonmotile depending apparently on whether they are separate or fused into the filaments.*

In suitable media the endospores begin to form the first or second day and finally become more abundant than the nonsporiferous rods.* The sporangia are short elliptical rods in which the endospore is borne centrally and fills nearly the whole body of the sporangium, the latter being swollen slightly around the spore.* Rarely the spore is borne at one end in a swollen portion. The sporangia measure 3 × 1.2 μ; the endospores measure 1.6 × 1 μ. The sporangia disappear by solution. The germination of the endospore is central, rarely polar. The flagella are three to four times as long as the rods: they are peritrichiate and eight to ten in number.* They were stained by Gaurnier's method. There is a thin capsule.

On agar the growth is abundant, spreading, lobed, flat.* It is glistening, smooth, becoming rugose, translucent, white to dirty yellow.

On glycerinated potato, growth is abundant, persistent, raised, transparent, white to dirty yellow,* gelatinous.

On glycerin agar, a dry pulverulent surface.

On sugar agar a very rapid spreading growth visible in four to eight hours, slime tenacious. Organism not much inclined to form acids.*

In agar stabs growth best at the top.* Surface growth restricted, lobed; no line of puncture.

Gelatin stab: Liquefaction funnel shaped, abundant at 20° C.*

Nutrient broth: Growth rapid, rim and pellicle present, the latter wrinkled, clouding moderate, fluid turbid, browned, sediment granular pulverulent.* Broth cultures often contain long, tangled chains recalling *Aplanobacter anthracis*.*

Milk is coagulated by a lab ferment and the extrusion of whey begins in two days.* Curd is peptonized in four or five days.

Agar colonies: Growth rapid, surface colonies round to lobed.*

The organism has lived nine months on culture media.* It is a strict aërobe.*

The optimum temperature for growth is 34° to 35° C.* Slow growth at 15° C. Grows well at 37° C.* Maximum temperature for growth 41° C. The spores are not killed by fifteen minutes at 102° C.

There is a slight production of acid on starchy media. Potato starch is destroyed.*

From the results obtained by Schiff we may assume the existence of at least three enzymes: Lab, trypsin, diastase.*

ADDITIONAL OBSERVATIONS BY THE WRITER.

To the foregoing I can add the following from my own studies:

The granules in the short rods stain deeply with carbol-fuchsin, becoming very pronounced. They are one to four or more in number. The length of the filaments from peptonized beef bouillon may be several hundred times their diameter and these long forms are not motile. They stain uniformly and deeply with carbol-fuchsin. Shorter filaments, however, were observed to be motile. The short rods taken from potato cultures were actively motile. I stained the flagella by Pitfield's method. They closely resemble the flagella of *Bacillus vulgatus*.

Intricate criss-cross marks are sometimes present in surface growths on agar. At other times the surface is smooth or finely granular under the lens. Copious wrinkling was readily obtained by adding cane sugar to the agar.

On glycerin agar a dry, white, rough, scaly growth was obtained. Growth on this medium was very abundant in the thermostat at 31° C. (50 times as much as at 20° C.)

In agar stabs the surface growth was thin, white, finely granular under the lens, finally spreading. A distinct stab growth appeared and this was best at the top.

The surface colonies on gelatin develop rapidly and are circular. The margin is fimbriate, as in the case of *Bacillus subtilis*, i. e., fringed with many parallel filaments. Next to this fringed portion in colonies two days old is a finely granular portion consisting of actively motile short rods. The center of the colony contains also tangled chains. These colonies measured 5 to 10 mm. in diameter and the gelatin was fluid except at the margin of the colony.

Gelatin stab: Growth gray white, best at the top; liquefaction rather rapid. It begins in about twenty-four hours at 24° C. and usually ends in five or six days. The tube of liquefaction is at first crateriform, becoming saccate.

A pellicle forms on the surface of milk. In litmus milk there was some formation of alkali but no distinct development of acid. The milk must therefore be coagulated by a lab ferment. In my hands the solution of the precipitated curd was not as rapid as described by Schiff.

In Petri-dish poured plates at 25° and 30° C. the growth of the surface colonies on + 15 agar is rapid. They are round, soon becoming irregular, rhizoid; the lobes are often branched repeatedly, as in the case of *Bacillus aroideae*, if the agar is not too dry. The surface is smooth or slightly roughened, gray white, edges entire, becoming variously lobate. Long, narrow crystals are sometimes found in agar cultures.

On potato with 5 per cent glycerin added, growth at first was white or gray white, becoming isabella colored, growth abundant, spreading, rhizoid, dull (shining under hand lens) rugose with dense angleworm-like folds.

Growth on common potato is similar, that is, a much wrinkled, rather dry looking, spreading surface layer develops quickly. This soon thickens and the numerous worm-like folds disappear, so that at the end of six or seven days the surface presents a smooth, thick, shining layer. The substratum meanwhile acquires a slight pinkish tinge or red specks appear here and there. The starch in the potato is destroyed and growth continues for some weeks. The medium remains neutral or with slight variations to either side of the litmus neutral point. The potato cylinders are softened; after six weeks only traces of starch remain.

In Uschinsky's solution the growth was copious but the rim was scanty. No growth was obtained in peptone water with 0.2 per cent

malic acid. No growth was obtained in oxalic acid agar stab cultures. No growth occurs in Cohn's solution.

No gas formation was observed; organism not tested in fermentation tubes.

Peptone water with dextrose becomes dark brown. A nutrient mineral solution (nitrogen-free media) with the addition of sodium asparaginate and cane sugar became dark brown; organism also able to take nitrogen from ammonium lactate, ammonium tartrate, ammonium citrate, and ammonium phosphate. Organism does not grow readily or copiously in distilled water containing only asparagin or in Cohn's solution with asparagin substituted for ammonium tartrate.

Nutrient agar with addition of cane sugar acquires a reddish brown stain; so does also Hunger's sugar agar using either monopotassium phosphate or dipotassium phosphate. Plain agar did not stain.

Boiled white of egg appears to be acted upon slightly.

Growth in nutrient broth, glycerinated peptone water, Uschinsky's solution, Fermi's solution, etc., is best at the top.

Nitrates are reduced. No indol in old peptone water cultures could be detected with sodium nitrite and sulphuric acid.

The organism lives a long time on culture media. So far as I have observed it is a strict aërobe: It grows feebly in bouillon under olive oil (six days), and in shake-agar cultures buried as soon as solid under another 10 c. c. of agar with olive oil on top, visible growth occurred only in the upper 10 c. c. of agar, and the colonies were largest in the upper strata of this agar (four to nine days).

In streak cultures on litmus agars the organism behaved as follows:

In each of 16 streak cultures on litmus agar there was a copious growth in twenty-four hours, but in forty-eight hours no reddening with plain agar, or the same with addition of lactose, galactose, maltose, mannit, or glycerin. With dextrose and saccharose, however, the litmus was feebly purpled, and this change was visible in twenty-four hours. At the end of seven days the plain litmus agar and that containing lactose and galactose were either slightly bluer or not different from the check tubes. The others were purplish. These litmus agars contained, respectively, dextrose, saccharose, maltose, mannit, and glycerin. None were bright red, only purplish red, and that not very pronounced, although plainly different from the check tubes. The litmus glycerin agar was reddened least, and this change was not visible until after the fourth day. There was no reduction of the litmus in any of the tubes (ten days). After seventeen and thirty days the litmus was reduced in tubes with glycerin. In addition to the carbon compounds to be tested this agar contained only filtered river water and Witte's peptone. We may therefore conclude that the organism is only a slight producer of acids.

When taken from very young bouillon cultures made from similar cultures, i. e., in such a way as to obtain spore-free material, the organism is readily killed by drying. Cloudy bouillon from such tubes when spread on glass cover slips and preserved in ordinary covered Petri dishes for from five to nine days in a closet at room temperatures failed to cloud tubes of bouillon when thrown into them.

That part of Schiff-Giorgini's paper distinguishing two distinct forms of olive tubercle—the primary, due to external infection, and the metastatic or secondary, developing from within—is well worked out and marks a distinct advance in plant pathology.

THE DISEASE IN THE FIELD.

It has been observed over and over again in Italy and elsewhere that rich soil, heavy manuring, and excessive use of water favor the spread of this organism, by producing a great quantity of juicy tissues suitable for infection. This is also true of pear blight. Some varieties of olives seem to be more subject than others. Very sensitive ones should be discarded.

Old tubercles are often eaten by various insects, and it is possible that some insect carrier plays a part in the distribution of this disease. This can be determined only by a prolonged investigation of field conditions supplemented by laboratory studies.

The skillful use of the pruning knife offers some hope. Special knives should be provided for the pruning of diseased trees, or else all the knives should be disinfected after each tree is gone over. In pruning it should be remembered that in some instances the organism may occur in the interior of the stem, some distance below the tubercle. It is wise, therefore, to cut several inches below visible signs. A dab of disinfectant on the cut surfaces, if they are not too numerous, is also to be recommended.

Of germicidal sprays, two may be recommended for trial on a small scale at first, i. e., until it is known definitely whether or not the foliage of the olive will be injured by them. These substances are Bordeaux mixture and the self-boiled lime-sulphur mixture recently devised by Scott for the treatment of peach diseases.[a] If the olive tree will bear either of these germicides, then thorough tests for the control of the disease should be made by giving a half dozen sprayings in the actively growing part of the season.

[a] See Circular No. 1, Bureau of Plant Industry, 1908.

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